Examining the Diagnostic Performance of Vaginal, Self-Screening for High-Risk Human Papillomavirus in Port-au-Prince, Haiti

by

Mariana Krueger

Duke Global Health Institute
Duke University

Date:_____

Approved:

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David Walmer, Supervisor

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Nahida Chakhtoura

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David Boyd

Thesis submitted in partial fulfillment of
the requirements for the degree of Master of Science
in the Duke Global Health Institute
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ABSTRACT

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Abstract

- **Background:** The incidence and mortality rates of cervical cancer in Haiti are among the highest in the world, yet the screening coverage rate is among the lowest. Efforts to ameliorate this problem using cytology-based programs have fallen short due to geographic, socioeconomic, cultural, and infrastructural barriers to access. A prevention strategy based on vaginal, self-screening for high-risk (HR) HPV in communities may increase coverage by avoiding or diminishing these impediments. This study examined the diagnostic performance of self-screening when compared to clinician screening in two clinics in Port-au-Prince.
- **Methods:** 1836 women participated in a cross-sectional study in which each underwent vaginal and cervical screening for HR HPV, and HIV rapid testing. HR HPV positive women returned for follow-up testing with colposcopy and biopsy. Data analysis explored the concomitant tests' comparative performance using percent agreements with Kappa statistics, test positivity, and the ability to detect various levels of biopsy-confirmed cervical intraepithelial neoplasia. Age-related prevalence rates were also determined. Statistical associations were measured using Chi-Square, Fisher's Exact, and McNemar's Tests.

- **Results:** Overall concomitant test agreement was strong (91.39%, K=0.73), but varied with statistical significance by age in the youngest and oldest quartiles. Women between 42 and 48 years old demonstrated the highest concordance (93.45%, K=0.70). Vaginal test positivity was uniformly higher than cervical test positivity among participants of all ages. Vaginal samples identified 84.46% of HR HPV cases that cervical samples identified, and more than 90% of all high-grade disease. However, clinician screening accurately detected several more clinically relevant cases of disease (≥ CIN I) (56) than self-screening (53).
- **Conclusion:** Strong test agreement indicated that vaginal screening produced comparable results to clinician screening, and age-related statistics may be able to inform test algorithms in the future. With plans to establish pathology labs in Leogane and Port-au-Prince that employ local talent and utilize the relatively affordable CareHPV Assay (QIAGEN), self-screening may be a diagnostically sound and financially feasible cervical cancer prevention strategy in Haiti.

Dedication

To the women of the world, in the hopes that the information presented in this document will help alleviate the global burden of cervical cancer.

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Acknowledgements

Thank you to Family Health Ministries, Duke University, and my fellow students for the opportunity to expand my understanding of the world.

1. Introduction

Cervical cancer, the seventh most common cancer in the world[1], is a disease of disparity. Both incidence and mortality are disproportionately greater among women living in low- and middle-income countries (LMICs) than those in high-income countries (HICs). Of the roughly 525,000 cases of cervical cancer that occur annually, 86% are in LMICs. Of the roughly 275,000 women who die from the disease each year, 88% are in LMICs [1]. Unfortunately, Haiti is a developing country emblematic of these statistics.

Haiti has the highest reported cervical cancer incidence rate (age-adjusted: 93.9/ 100,000) and mortality rate (age-adjusted: 53.5/ 100,000) in the Americas[2]. By the year 2025, the International Agency for Research on Cancer (IARC) estimates that as the population grows and ages, the annual number of cervical cancer deaths in Haiti will be 55% higher than in 2008 [1]. This is a devastating projection, given that cervical cancer already accounts for 49% of all cancer deaths in the entire population[3], and 63% of all cancer deaths in women[4].

It is possible to reverse these numbers, however, with the implementation of a national cervical cancer prevention program in Haiti. Decades of research from the United States and Western Europe have demonstrated that cervical cancer incidence and prevalence can be halved through the inauguration of regular screening practices that reach a high percentage of the population. While cytology is the current method of choice in high-income countries, it has routinely failed to have a positive impact in low

and middle-income countries where infrastructure is weak, skilled pathologists are few, money is scarce, misconceptions are rampant, and transportation is difficult. In Haiti, an inaugural, cytology-based screening strategy struggled with many of these issues, as well as the high prevalence of reproductive tract inflammation. Family Health Ministiries (FHM), the non-governmental organization (NGO) that piloted the program, discovered obscuring inflammation on 85% of cervical cytology slides from women who had been pretreated with antibiotics for clinically-evident vaginal infections[5].

Visual inspection with acetic acid (VIA) has also been employed in many developing countries in the hopes of countering cost and reducing the need for skilled labor. However, research suggests that VIA's quality assurance and sensitivity results in significant numbers of missed cases of disease. Most notably, in a large, randomized cluster, controlled trial in India[6] of more than 130,000 women, clusters of villages that underwent either cytology or VIA experienced no significant reductions in the number of advanced cervical cancer cases or associated mortalities relative to the control group, which received the standard of care. In contrast, those women who received HPV testing showed significantly lower incidence and mortality rates. Given these compelling findings, we decided to explore patient-collected HPV testing as a potential primary screen for cervical cancer prevention in Haiti.

Self-performed HPV screening has the potential to increase Haiti's screening coverage (last measured in the 1980s as approximately 5%[7,8]) by overcoming barriers to

access. Many studies from around the world, including from "Little Haiti," a bastion of Haitian immigrants in Miami, have demonstrated overwhelming acceptance of, and often preference for, vaginal self-sampling for HPV. Research has also shown that this method of screening is diagnostically sound when compared to the highly sensitive and specific method of clinician-performed HPV screening. However, some variation in the strength of agreement has been observed, and some postulate that the testing instrument, size or location of the sample, detection target (high-grade or low-grade disease), and population characteristics can offer insight into the discrepancies[9,10]. Most notably, it has been established that HPV DNA test performance is affected by the prevalence of HPV, which varies by age[11–13]. Despite this awareness, current manufacturers of the Hybrid Capture II Technology (hc2) (QIAGEN Corporation, Gaithersburg, Maryland) – the most widely used HPV DNA test in clinical settings – recommend an interpretive algorithm that is the same for all women[14].

To understand the suitability of HPV self-screening to the Haitian context, it is important to determine not only the overall concordance of the concomitant tests, but also to probe the tests' performance in women with different ages, levels of cervical intraepithelial neoplasia, and in the future, HPV viral loads. Understanding these relationships can help optimize diagnostic performance and influence implementation strategies. In a country starving for health statistics, exploring these topics could provide valuable insight for clinicians and politicians alike.

2. Study Objectives and Hypotheses

With the gravity of cervical cancer and the lack of available screening in mind, this study pursued two objectives: 1) to determine if self-screening for high-risk (HR) HPV is a suitable cervical cancer prevention method in Haiti based on its diagnostic performance; and 2) to lay a foundation of age-related data that can be used to understand results and optimize test sensitivity and specificity in the future. Several hypotheses accompanied each of these objectives.

In assessing the primary objective:

- Self-collected, vaginal samples' results will agree with clinician-collected, cervical samples' results in at least 85% of patients.
- Self-screening will identify at least 85% of all cases of HR HPV that would have been identified by clinician-performed screening alone.
- Self-screening will identify at least 85% of histologically-confirmed cases of ≥ CIN I and ≥ CIN II.

In assessing the secondary objective:

- HR HPV prevalence, measured by both vaginal and cervical screening, will vary with statistical significance by age quartile.
- Self-screening and clinician-screening will return statistically similar results ($p > 0.05$) in each age quartile.

3. Thesis Structure

To best accomplish these objectives, this thesis is divided into several sections that intend to fully explain the research's context, execution, and future application. First, a review of the relevant literature describes the determinants of cervical cancer, the barriers developing countries face in screening for the disease, the reasons for choosing self-screening over alternatives, and HPV tests' diagnostic performance in self-collected samples. Next, the study's setting, clinical procedures, and methods of analysis are described in detail, followed by some of the methods' limitations. The results of the quantitative analysis then appear in both written and tabular form. In the order in which the research objectives and hypotheses were first presented, the results are discussed in the context of previous literature. A conclusion section then summarizes the findings and suggests their implications for the future research.

4. Background

4.1 Determinants of Disease

The two major determinants of cervical cancer incidence are persistent, carcinogenic (or "high-risk") human papillomavirus (HPV) infection, and lack of access to screening [15]. Notable risk factors for cervical cancer include: sexual activity at an early age, multiple sexual partners, exposure to other sexually transmitted diseases, cigarette smoking, oral contraceptive use, human immunodeficiency virus (HIV), and immunosuppressive drug therapy[16]. While cervical cancer is associated with these factors, it is important to note that HPV, the virus that leads to nearly 100% of cervical cancer cases, is fairly ubiquitous among the human population. For example, by the age of 50, 80% of sexually active women in America will have acquired an HPV infection at some time in their lives[17].

4.1.1 High-Risk Human Papillomavirus Infection and Persistence

Of the roughly 100 phylogenetic types of HPV, over 40 infect the basal or mucosal epithelial cells of the male and female genitalia and oropharyngeal tracts. These are classified as "low-risk" (LR) or "high-risk" (HR) based on their oncogenic potentials. LR HPV can cause warts, while HR HPV can cause cancer over time—typically years or decades after initial infection[18,19]. Anogenital transmission mainly

occurs through skin-to-skin or mucosa-to-mucosa contact [20,21], but the precise mechanism of pathogenesis is unknown.

According to the cervicovaginal challenge model, viral infection occurs via uptake and internalization. First, the small HPV virus bypasses the squamous epithelial layer of the transformation zone (a transitional area of the cervical canal where glandular epithelia are replaced by squamous epithelia) via a microabrasion that exposes the basement membrane. Second, after undergoing conformational changes achieved through antigen binding and cleavage, the virion is recognized by cellular receptors on the basal target cells, allowing for internalization [22]. By activating cellular DNA replication factors, widespread infection of the epithelia can occur within two to three days [20,23].

While the vast majority of infections—particularly those in immuno-competent women younger than 25—are cleared or suppressed within two years by cell-mediated immunity, some endure and even advance towards cervical cancer [24]. Dysplastic cellular changes in the transformation zone create lesions known as "Cervical Intra-epithelial Neoplasia" (CIN) or "Squamous Intraepithelial Lesion" (SIL). Typically, 43% of low-grade SIL ("LSIL," or CIN I and II) and 92% of high-grade SIL ("HSIL," or CIN III) either persist or progress [25]. Of the aggressively persistent HPV infections that develop into cancer, 80 to 90% are called "squamous cell carcinomas," because they

develop within the squamous cells of the ectocervix. The remaining 10 to 20% of cases are adenocarcinomas, which occur in the columnar or glandular cells of the endocervix[26].

Research has exposed 13 HPV types as the culprits of these persistent precancerous infections, HSIL, and cervical cancers —16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82. Although geographic differences do occur, the most prevalent types reported worldwide are 16 and 18, which account, respectively, for 20-30% and 50-70% of invasive cervical cancer cases in symptomatic and asymptomatic women worldwide[18,23]. Data collected by Family Health Ministries' clinics in Leogane and Port-au-Prince indicate that the most prevalent HR HPV types found in both single and multiple infections among 269 Haitian women were: 52 (11%), 16 (11%), 18 (10%), 35 (8%), and 31 (7%). HPV16 and HPV35 were the most common genotypes detected in ≥ CIN II, and both were found more often in women with high-grade disease than those without. Consistent with other global findings, HPV16 and/or HPV18 were detected in 21.0% of CIN II (n = 42), 46.2% of CIN III (n = 52), and 80% of cancers (n = 5). These statistics, while preliminary, suggest that the currently available bivalent and quadrivalent HPV vaccines currently on the market—which target types 16 and 18 and 6, 11, 16 and 18, respectively— may be less effective at reducing cervical cancer incidence and mortality in Haiti than in other parts of the globe[27]. This possibility underscores the importance of screening in preventing cervical cancer.

4.1.2 Lack of Cervical Cancer Screening

Despite Haiti's high incidence rate, the country lacks a national cervical cancer prevention program, and one estimate suggests that only 5% of at-risk women are screened[7,8]. This coverage rate is similar to those reported in Malawi, Ethiopia, and Bangladesh. In stark contrast, the percent coverage rates in many developed countries are in the mid-90s[28]. Over the last few decades in certain HICs, effective screening practices have led to dramatic reductions of between 50 and 80% in cervical cancer incidence and mortality[1,29,30]. Specifically, incidence in Finland decreased by roughly 80% between 1962 and 1993, while mortality dropped by 75% in the United States between the 1960s and new millennium[30,31]. The impact of screening on incidence and mortality is evident; however, program designs and implementation strategies that have proven effective in developed countries are not necessarily transposable to developing countries like Haiti, where the barriers to access are many and varied.

4.2 Barriers to Access in Developing Countries

Cervical cancer screening is impeded in LMICs by structural and intrapersonal factors that are less common in HICs. Varughese and Richman (2010) identify low literacy, culture and religion, competing health needs, poorly developed health systems, limited information on cancer prevention, lack of infrastructure, and geographic

isolation (which incurs time and transportation costs) as impediments to cervical cancer screening in developing countries[32].

Country data from prevention programs in Latin America and the Caribbean, summarized in a 2004 report by the Pan-American Health Organization (PAHO), exemplify these barriers. Specifically, studies from Costa Rica, Peru, Mexico, and Bolivia indicate wide intra-country variations in cervical cancer incidence based on women's geographic isolation, poverty, and lack of education. Cytology-based screening programs in the region have also reported resistance to participation because of patients' attitudes towards screening, diagnosis, and treatment. Many women report that the impersonal manner and/or male gender of clinicians deter them from having a pap smear taken. Others are fearful of cervical cancer diagnosis and treatment, believing that cervical cancer is a death sentence[33]. It is therefore evident that the reasons belying poor coverage rates in parts of Latin America and the Caribbean are multifaceted and entangled in complicated development and sociocultural issues.

The paucity of research conducted on this subject in Haiti corroborates the findings from elsewhere in the region. In personal communication (May 25, 2012), Drs. David Walmer and Nicole Tinfo of Family Health Ministries (FHM) cited transportation costs and geographic barriers as reasons for a 50% loss to follow-up in a cytology-based screening program in Leogane, Haiti, which ran from 2009 – 2011. In her Master's thesis (2011), Marie Hilaire also concluded, through univariate and multivariate analyses of

five cervical cancer predictor variables from the Haitian Demographic and Health Survey of 2005-2006, that pecuniary and geographic obstacles were correlated with cervical cancer (and by proxy, with screening). Hilaire found that both crude and adjusted logistic regression models demonstrated that women with low educational attainment and socioeconomic status have increased odds of developing cervical cancer compared to the referent group[8]. The crude model also pegged rural residence as a risk factor. It is important to note, however, that three dependent variables of questionable suitability were used as proxies for the outcome of interest, high risk of cervical cancer: young age (less than 20 years old) of first sexual intercourse, multiple lifetime sex partners, and inability to ask partner to use a condom. The author describes neither the biological nor socio-cultural reasons for selecting 20 years as an age of significance in the development of cervical cancer. Furthermore, the "condom variable" falsely equates women's ability to ask for protection with reduced or negligible exposure to HR HPV. Asking does not ensure utilization; utilization does not ensure accurate utilization for the duration of every sexual encounter; and even perfect utilization does not ensure protection from HPV transmission.

While Hilaire's thesis contains methodological shortcomings, it represents the only known research of its kind in Haiti, and therefore is cautiously considered evidence of cervical cancer's connection to poverty, lack of education, and rural residency in Haiti. Until robust statistics are available, Haitian policymakers and public health

officials must review the successes and failures of other countries' battles against cervical cancer to inform their own country's cervical cancer prevention program.

4.3 Assessment of Cervical Cancer Screening Methods

In general, the most impactful programs—those that effectively reduce the incidence and mortality of cervical cancer by detecting and treating precancerous lesions— employ tests that are relatively simple, inexpensive, sensitive and specific, painless, and socio-culturally acceptable. They can be applied to large numbers of asymptomatic people in order to classify them as likely or unlikely to have cervical dysplasia, and women with positive results can then be further investigated or treated[34]. Many different screening techniques have been employed in LMICs with varying success. An examination of these techniques supports the potential suitability of vaginal self-screening to the Haitian context.

4.3.1 Cytology

Cytology, also known as the Papanicolaou test or pap smear, is likely the most widely used cervical cancer screening technique in the world[35]. In high-income countries with robust health infrastructures, broad screening coverage, and reliable patient follow-up, conventional and liquid-based cytology has diminished cervical cancer incidence and mortality dramatically. In the United States, pap smears were introduced in 1945 and are still used as the primary screening method for women aged

21 and older. Unfortunately, cytology-based screening programs in low-income countries have floundered. In Latin America and the Caribbean, specifically, documented problems with cytological screening include the low quality of smear sampling, collection, preparation, and interpretation[33]. These problems are traceable to three sources: high cost, reliance upon skilled labor, and weak sensitivity of detecting HSIL and cancer[32,36].

Cytology screening is expensive because of the requisite equipment, skilled labor, and infrastructure. Tools are needed for sample collection (e.g. speculum), processing (e.g. slide stains), and interpretation (e.g. microscopes). Highly-trained nurses or doctors are needed to perform pap smears, while cytotechnologists and pathologists are needed to render quality-assured diagnoses. In addition to the physical infrastructure necessary to conduct clinical and diagnostic functions, cytology-based programs require organized, programmatic infrastructure that enables patient tracking and adherence to follow-up[36]. Maintaining patient registries and contacting women for test results, colposcopies, and treatment are salient to this method's success, because they ensure repetitive screening and relatively short testing intervals.

Frequent testing and follow-up visits are needed because when used on its own, cytology has a relatively low sensitivity. Meta-analyses and recent, large studies have demonstrated that the sensitivity of a single pap smear is between 49% and 57% for HSIL and cancer[36]. In countries where women are screened only once or twice during

their lifetimes, if at all, this sensitivity range is not strong enough to mitigate the national burden of disease. Assuming 100% of women are screened, but only once in their lifetimes, using pap smears alone would prevent over 40% of women with HSIL and cancer from receiving accurate diagnoses and treatments. It has been suggested that combining cytology with a second test would increase sensitivity while reducing testing intervals; however, the cost-effectiveness of this strategy remains to be established[34].

Using pap tests to correctly identify women with HSIL is especially challenging in the presence of cervical tissue inflammation, which can obscure malignant cells and lead to high rates of false negative diagnoses[37]. While inflammation is often caused by benign conditions like vaginal infections, it is associated with a number of sexually-transmitted infections and malignant neoplasia[37–41]. In fact, data from Hammes and colleagues (2007) in Brazil demonstrated a direct, linear relationship between inflammation and disease progression; inflammation was present in 25%, 46.1%, 58.4%, and 89.3% of benign tissue, LGSIL, HGSIL and squamous cell carcinoma, respectively[42].

The connection between inflammation and false negative results are especially important in Haiti where the prevalence of the former is high, and the consequence of the latter is dire. Between 2009 and 2011, gynecologists performing pap smears at two FHM clinics in Haiti observed obscuring inflammation in as many as 85% of cervical specimens[5]. While uninvestigated at this point, researchers from the University of Miami have hypothesized that the inflammation is related to a high frequency of vaginal

cleansing (two to three times daily) using both natural and commercial products[43]. This practice, performed in many parts of Africa as well, is intended to dry and tighten the vagina for men's sexual pleasure[44,45]. While liquid-based cytology is believed to have increased sensitivity over conventional cytology in cases of inflammation[46], its benefits are potentially nullified by its relatively higher cost[36]. Another Duke Masters in Global Health student, Genevieve Wolpert, is currently investigating this possibility in Haiti.

4.3.2 Visual Inspection with Acetic Acid or Lugol's Iodine

Visual inspection techniques rely on visualization of the cervix under bright light at least one minute after the application of either acetic acid or Lugol's iodine, which increase the color contrast between healthy tissue and areas of acetowhitening, where cervical dysplasia may exist. In low-resource settings, these techniques come with both advantages and disadvantages when compared to other screening methods.

The main advantage of visual inspection with acetic acid (VIA) over cytology is its simplicity; it is easy for health workers and convenient for patients. After roughly a week of training, health workers from a variety of backgrounds are able to competently screen women. Results are nearly immediate, so health workers can diagnose and treat qualifying patients in a single visit. With a pooled, average sensitivity of 80% and specificity of 92%, based on meta-analysis of 26 studies of asymptomatic women in mostly developing countries, VIA generally offers improved power of detection when compared to cytology[47]; however, its performance varies widely.

A review of 11 studies conducted by Sankaranarayanan and her colleagues at the International Agency for Research in Cancer (IARC) in 2004 found that VIA's sensitivity and specificity were highly irregular in different settings, despite commonalities among patient populations and clinician training methods. For example, VIA's sensitivity for CIN III in Kolkata and Mumbai was 58%, while in Burkina Faso and Guinea, it was 90.3%. Similarly, specificity for CIN III varied widely between Jaipur and Congo: 76.6% vs. 93.8%[36].

The inherent subjectivity of the screening process explains these large disparities. In a cross sectional study from India, a master trainer and test providers only agreed in 64.5% (K = 0.38) of diagnoses, which is indicative of fair agreement[48]. To improve test performance, some have suggested that quality control might be improved by using magnification or Lugol's Iodine instead of acetic acid. Indeed, VIA with magnification (VIAM or colposcopy) is considered an enhanced version of VIA; however, cross-sectional studies from India and South Africa have suggested that magnification does not necessarily improve test performance over naked eye assessment[49,50]. On the other hand, visual inspection with Lugol's Iodine (VILI) does garner more sensitive (77.8 – 98.0%) and specific (73.0 – 91.3%) results than VIA, based on research from 10 study sites with 49,000 women[34].

Despite VILI's apparent advantages over VIA, available research indicates that only the latter is currently practiced in Haiti. In 2013, DirectRelief launched a pilot VIA

program in Port-au-Prince using locally-trained clinicians, screening 600 women in the first week[51]. To date, research has yet been published on the program's performance.

4.3.3 Clinician-Performed HPV Testing

HPV testing, first used in a clinical capacity in the late 1980s, is a relatively modern screening approach that has gained the attention of researchers and public health officials for its successes as both a primary and supplemental screening method. Currently, the United States employs reflex-HPV testing in women with pap smears that reveal atypical cells of undetermined significance, while many European countries are exploring the possibility of using HPV testing, rather than cytology, as a primary screening tool[52]. Unlike other approaches, which focus on the visualization of dyskaryotic cells at either the microscopic (cytology) or macroscopic (colposcopy) levels, HPV testing detects the causative agent of cervical cancer sometimes before visible, high grade neoplasia has developed. Through polymerase chain reaction (PCR) or Hybrid Capture 2 (hc2) technologies, viral copies of DNA in cervical samples register a "positive" test result when present in numbers above a certain threshold. This threshold can be adjusted according to the specificity and sensitivity needs of the population[53].

HPV testing is especially useful in LMICs because of its high sensitivity, negative predictive value, reproducibility, and quality assurance[6]. Compared to cytology and VIA/ VIAM/ VILI, HPV tests' sensitivity for detection of ≥ CIN II is markedly elevated[52]. In a recent meta-analysis of 22 studies from diverse settings, Cuzick, Arbyn, and

Sankarayanan et al. (2008) found that HPV tests' pooled sensitivities for ≥ CIN II and ≥ CIN III were 93.1% and 95.5%, respectively[54]. Since that meta-analysis was conducted, several more studies have been published confirming physician-collected cervical samples' ability to detect high grade disease with greater than 90% sensitivity [9,55,56].

Research from developing countries exemplifies the benefits of HPV tests' sensitivity. Most notably, in a large, cluster-randomized control study of more than 131 thousand asymptomatic, Indian women from 497 villages in Osmanabad, a single round of HPV testing was associated with significant declines in the rates of advanced cervical cancer and cervical cancer-induced mortality. Contrastingly, neither VIA nor cytology evinced positive results when compared to the control cluster of 13 villages that received the standard of care. Specifically, the age-standardized rate of invasive cervical cancer among those with negative results from VIA or cytology was four times as high as the rate among HPV negative women[6]. One meta-analysis from 2008 concluded that overall, as a follow-up test to a positive pap smear, the hc2 assay is 14% more sensitive than cytology, but equally as specific[54]. This disparity in sensitivity is explained in part by HPV tests' ability to detect pre-cancer and cancer at earlier stages of infection than other screening methods[57]; in fact, about two-thirds to three-quarters of positive HR HPV cases are accompanied by pap smears showing no cellular abnormalities[58].

In addition to being very sensitive, HPV tests are also strong predictors of the absence of disease when compared to other screening methods. A high negative

predictive value (NPV) ensures that women who receive negative HPV results are not only negative for high-grade dysplasia, but also are unlikely to be infected with HPV. Several studies from around the world have compared HPV testing's NPV to VIA's and cytology's, and each has concluded that HPV testing outperformed the rest. Negative predictive values for ≥ CIN II consistently range between 95% and 100%; only among HIV positive women in South Africa did the NPV dip slightly to 94.1%, though this value was still better than those for VIA (87.5%) or cytology (89.7%)[56,59–61]. Because of the method's reliably strong negative predictive value, many researchers have suggested that screening intervals could be lengthened from the three to five years (used in cytology) to six to ten years[62–64].

High sensitivity and negative predictive values in primary screening are particularly important in developing countries where women have infrequent interactions with health systems, and therefore may only undergo screening once or twice in their lifetimes. Additionally, the extended screening interval could significantly reduce physicians' patient burden and funders' financial burden. Utilization of QIAGEN's CareHPV assay, a cheaper, easier, but equally accurate alternative to hc2 that gives results in three hours, could also help drive down costs[53].

HPV testing's reproducibility augments these characteristics by providing superior quality assurance to other methods; a multi-center, multi-day study found that hc2 test results agree 99.5% of the time (Kappa = 0.990)[6]. The three participating

laboratories were given identical reproducibility panels of HPV DNA targets, HPV positive clinical specimens, and HPV negative clinical specimens. 100% of expected positive specimens returned positive results and 99% of expected negative specimens returned negative results[14].

Using specified calibrators and an HR HPV control sample of 5 pg/mL (500,000 copies/mL) of cloned HPV 16 DNA and carrier DNA ensures each test's quality. Digene (now QIAGEN) computer software, rather than human eyesight, determines the calibrators' and controls' positions on the microplate[14]. With the help of advanced technology, training technicians to perform HPV testing is less demanding than training cytologists.

For all its benefits, HPV testing can still be a challenge to implement in some low-resource settings, for a couple of reasons: namely, its utilization of clinicians and need for existing infrastructure. While individuals who complete short training courses can perform hc2 testing, physicians or nurses are required to collect cervical specimens. With a need for high coverage rates, reliance upon already over-burdened clinicians for screening is challenging in countries like Haiti, where the physician to population ratio is 3/ 10,000[65]. This led us to test the hypothesis that self-performed vaginal sampling may be as effective as clinician-performed cervical sampling in identifying disease.

4.4 Self-Performed HPV Testing

Self-screening for HPV and other sexually-transmitted infections was first suggested in the late 1970s, but has only recently gained global traction as an alternative testing method for women in medically underserved areas[45]. Interest has burgeoned as research has demonstrated self-screening's strong diagnostic performance relative to other methods and its widespread acceptability among diverse populations of women. While the sensitivity of self-collected specimens is high using most tools (cytobrushes, cervicovaginal lavages, tampons, and Dacron swabs), studies have shown that cotton swabs are infeffective[66,67]. Brushes appear to have the greatest sensitivity of detecting ≥ CIN II, and are therefore the standard tools that accompany QIAGEN's cervical sampler kits.

4.4.1 Variations in Tools, Techniques, and Testing

At present, there are no standardized instructions for self-sampling, which may explain some of the variations in sensitivities and specificities observed in different studies. Some postulate that sample accuracy is dependent upon proximity to the cervix; others focus on the size of the sample collected, which might be influenced by position, instrument, or the number of rotations of the sampling device[9,10]. Among women reluctant to collect their own samples, concern over incorrectly performing the test is the most commonl complaint[10]. With public health officials increasingly considering self-sampling as a viable screening tool, particularly in rural and

impoverished areas, scientists and policy-makers ought to develop and approve universal instruments and instructions for their use.

There is also some disagreement, or at least variation, among the cut-off points for positivity on the most widely used assays: hc2 and CareHPV. Some researchers, using ROC curves, have adjusted the relative light units' (RLU) proportion to the cut-off (CO) value according to populations' needs, either raising or lowering the value from QIAGEN's prescribed "1.0"[53,68]. These adjustments allow scientists to address one of the main complaints against HPV testing: its diminished specificity relative to cytology. By increasing the RLU/CO, specificity can be increased to exclude samples with weakly positive signal strengths, and the opposite can be done to increase sensitivity[69,70]. Public health officials, who must weigh the consequences of increasing physicians' patient loads or denying colposcopies to women who may never be screened again, can benefit from this flexibility.

Regardless of variations in methodologies, studies consistently report that self-screening is both diagnostically strong and well-liked by participants in home and clinical settings[10,49,71–73].

4.4.2 Diagnostic Performance

The findings from a number of studies from around the world have contributed to the understanding that vaginal self-sampling is a diagnostically capable method of cervical cancer screening when compared to alternatives. In a review of 28 studies from

1992 to 2012, Snijders et al. (2013) cogently presented the evidence for this, though not all 28 were included for consideration in this discussion. Studies that calculated sensitivity and specificity on the basis of available histology— only from women with positive HPV tests or abnormal paps— did not account for verification bias by randomly sampling a handful of HPV-negative or "normal pap" patients. Therefore, only research papers that addressed this bias through random sampling or universal application of a reference standard are mentioned hereafter.

Of five studies that examined the accuracies of self-collected specimens to physician-performed pap smears, three of them showed that the former was better able to detect ≥ ASCUS or ≥ LSIL than the latter[55,56,74]. In these cases, the pooled sensitivity for self-screening was 86.3%, compared to cytology's 79.6%. Including the two studies in which cytology (one conventional and one liquid-based) was more sensitive than self-screening, the overall pooled sensitivity for the five studies was 84.5% for HPV testing and 85.2% for cytology [10,75,76].

Interestingly, according to the five studies of referral and healthy populations highlighted in Snijders et al. (2013), HPV testing and pap smears performed equally well in specificity for ≥ CIN I; the pooled specificity was 69.4% for HPV self-screening and 67.3% for cytology. It is possible that both measures of diagnostic performance were comparable because low-grade disease was used as the detection target rather than high-grade disease, or because the prevalence of disease was low in the tested

populations. When researchers have established ≥ CIN II as the target for detection, self-samples' sensitivities have been mostly recorded in the 90s, and even as high as 100%, while specificities are slightly lower, typically ranging between 70 and 90% for healthy patients, and 50 to 70% for referral patients[10,77,78].

Relative to clinician-performed HPV testing – the most sensitive screen for high-grade disease available— self-sampling fares remarkably well. To measure vaginal samples' diagnostic ability against cervical samples', researchers often compare the concomitant samples using percent agreement and an accompanying Kappa statistic, K. Percent agreements evince concordance while Kappa statistics describe the strength of that concordance (on a scale from -1 to 1) relative to what one would expect to garner from chance alone[79]. In the past decade, several meta-analyses have examined dozens of studies comparing the diagnostic performance of self-sampling to physician sampling, and each analysis has concluded that the preponderance of studies demonstrate that the tests bear high percentages of agreement with moderate (0.41 – 0.60) to good (0.61 – 0.80) Kappa values[80–83].

Importantly, results appear uniformly positive in both high-income and middle- to low-income countries, and in both healthy and referral populations. In two studies from Munich in 1999 and 2004, concordance rates between self-collection and physician-collection were 83 and 92% (K=0.71), respectively[64,84]. Researchers found that cases of test disagreement were mostly due to vaginal positivity and cervical negativity.

Hillemanns et al. (1999) demonstrated that while self-collected and clinician-collected samples were equally sensitive (92%) for high-grade disease, the former was more sensitive at detecting histologically-confirmed CIN I. Research from South America, Asia, and Africa has produced similar results with Kappa values all greater than 0.7 [56,85–87]. In women greater than 18 years of age attending a community health center in Gugulathu, South Africa, Jones et al. (2007) only garnered such strength of agreement when using the Roche Reverse Line Blot Assay (RLBA) (Roche Molecular Systems Inc., Branchburg, NJ, USA) to determine results. Using swabs as the collection device and RLBA as the testing technology, vaginal and cervical samples showed 89% concordance (K=0.75). Using hc2 assay, swabs were 81% concordant (K=0.61) and tampons were 82% concordant (K=0.55). These results, while all indicative of moderate to good agreement between HPV tests, evince that many variables, including technology, can affect diagnostic performance.

4.4.3 Age and HPV Prevalence

Some research suggests that the relationship between age and HPV DNA test diagnostics is mediated, at least in part, by varying HPV prevalence rates among different age cohorts[11,13,88–92]. In two large, population-based studies in China (n = 28,848 and n = 13,004), researchers revealed that in both physician-collected, cervical specimens and self-collected, vaginal specimens, specificity decreased with increasing age; those younger than 35 years old had the highest specificity[91,93]. Other reports from Europe and

North America contradict these results, demonstrating greater specificity in older women[94]. These incongruent patterns are possibly reflective of disparate age-related prevalence rates in the two regions: HPV peaks only in young women in North America and Europe[90], but peaks both in young and middle-aged women in China[93]. Global disparities can be quite significant; in fact, a large, cross-sectional study of 15 areas from four continents indicates that age-specific prevalence curves sometimes vary by an order of magnitude between locations.

Recently, Elkins et al. (2013) substantiated previous evidence linking patient age with test performance while displaying the importance of RLU/CO values in rendering HPV diagnoses. Using 359 cervicovaginal samples that produced initially equivocal hc2 test results (RLU/CO between 1 and 2.5 pg/mL), researcher found that re-test results were dependent on age. As age increased, the number of true positives (RLU/CO $\geq$ 1) decreased: 93.3% of 15 – 29 year-olds received secondary positive diagnoses compared to 71.9% of women 50 and older[13]. Another study of women with weakly positive hc2 tests revealed that when the RLU/CO value was set anywhere between 1 and 10 pg/mL, no cases of CIN II or greater were discovered in women over 50[92].

Certain low-risk (LR) HPV strains, that are known to sometimes cross-react with the hc2 assay to produce false positives, also fluctuate in prevalence by age[14]. These geographic inconsistencies in age-related prevalence rates and test performances

underscore the need for age-stratified data in Haiti, as they could, among other things, inform RLU/CO adjustments and recruitment targets.

4.4.4 Cost

While most HPV testing is relatively expensive and requires skilled laboratory services that are uncommon in LMICs, vaginal self-screening in Haiti may prove to be cheaper than alternatives for several reasons. First, QIAGEN and PATH recently developed a DNA assay called "CareHPV" that promises to be a cheaper and simpler alternative to the currently available commercial assays [53]. Second, QIAGEN and Family Health Ministries are in the process of training Haitian lab technicians and building pathology labs in Port-au-Prince and Leogane that are intended to accommodate the HPV testing needs of thousands of women. Third, recent data from five different developing countries evinced that clinician-performed HPV DNA testing is more cost-effective than conventional cytology for preventing cervical cancer in those settings. With a high sensitivity and negative predictive value, screening for HPV allows for greater time intervals between sampling. Self-screening, if equally as sensitive as clinician-performed screening in Haiti, could also reduce sampling frequency and overall cost relative to cytology[95,96].

4.4.4 Cultural Acceptability and Attendance Rates

In addition to its diagnostic and potential financial advantages over cytology and VIA, HPV self-screening is also widely accepted and preferred over more intrusive

methods by patients. Indeed, women from urban and rural settings, rich and poor countries universally respond positively to self-testing. Data from around the world have shown comfort or acceptability rates ranging from 82% in Kenya to 98% in Mexico [45,64,71,72,84,97]. Preference over other methods is also strong in a variety of settings: 97% of German internal medicine outpatients and 93% of Greek gynecological patients expressed partiality towards self-screening rather than cytology. How these high approval ratings will impact attendance rates is still largely unknown, however.

According to a comprehensive meta-analysis of the literature from 1992 to 2012, only 9 studies have measured the impact of self-screening on attendance rates among non-attendees of regular screening programs. These studies, all completed in developed countries, showed improvements in attendance (typically by mailing-in samples) of between 8.7% (in Italy) and 39.1% (in Sweden)[10]. Although similar statistics do not exist for programs in LMICs, one study from rural Brazil provides encouraging evidence for community health workers' ability to increase screening coverage among non-attendees. After just one day of training, community health workers (CHWs) successfully recruited 878 women by going door-to-door. According to Holanda et al. (2006), 100% of women approached by CHWs agreed to perform an HPV test, and impressively, 100% of women went into the clinic a week later to undergo follow-up testing[87]. Training CHWs in Haiti to match this success could allow for a miraculous reduction in disease through increased screening coverage.

While research has not yet been published on the cultural acceptability or preferences of self-screening in Haiti itself, data collected from Haitian immigrant women (97% of whom were foreign-born) in Little Haiti, Miami, suggests the method would be well-tolerated. Kreyol-speaking, female community health workers (CHWs) of Haitian descent recruited adult Haitian women from various venues where they established a later meeting time to perform the tests in places of the participants' choosing (usually their homes or friends' homes). Of the 246 who participated, 242 gave adequate specimens after receiving verbal and pictorial instructions from a CHW. 95.1% of women found the self-collection device ("Fournier," which has the appearance of a tampon) easy to use, and 97.6% found it comfortable to use at home. Additionally, 98.4% of participants responded that they would recommend this test to friends and family members, and 87% said they preferred it to pap smears[45].

To explain this positive response, the researchers behind the study cited anecdotal evidence and previous ethnographic fieldwork. Their sources attribute the overwhelming acceptance of, and preference for, self-sampling to: the lack of pain experienced, cultural conceptions of modesty and privacy, discomfort with male clinicians, and concern about vaginal tone. Maintenance of vaginal tone or tightness is especially important to Haitian women, who cleanse themselves multiple times a day as part of the effort (Barbee et al. (2010) believe this practice to be the source of inflammation seen in over 45% of Haitian immigrants' pap smears). Some women fear

that introducing a speculum into the vagina to perform VIA or pap test could make them too "open," causing their partners to suspect infidelity or express dissatisfaction during intercourse[98]. This testimony conveys the complexity of selecting an appropriate cervical cancer prevention strategy in Haiti; while diagnostic performance and cost may be important considerations for clinicians and government officials, cultural beliefs are salient to women's health decisions.

5. Materials and Methods

5.1 Study Setting

Two clinics in Port-au-Prince, Haiti served as the testing locations for this study: Family Health Ministries' Blanchard Clinic and Centre Haitien d'Investigation de Traitement Avancé de L'Infertilité (CHITAI). The overwhelming preponderance of participants were tested and treated at the modest, two-storied Blanchard Clinic, which is nestled on the grounds of a compound that also includes a primary school, Protestant Church, and guest quarters for visiting missionaries and medical teams. The surrounding neighborhood, Terre Noire, is one of the poorest areas within the capital city. Contrastingly, CHITAI is a private, fertility clinic that typically caters to patients within the relatively affluent community of Petion-Ville, home to foreign diplomats and local government officials.

5.2 Research Design

From May 2012 to April 2013, 1836 women participated in a two-part, cross-sectional study conducted at two clinics in Port-au-Prince, Haiti. The first part of the study, which served as the basis for the research presented in this document, consisted of: 1) self-performed and clinician-performed HR HPV screening; 2) visual inspection with acetic acid magnified (colposcopy); and 3) cervical biopsy of precancerous lesions. As of April 2013, colposcopies and biopsies are ongoing. The second part of the study, which is also ongoing, examines the accuracy of colposcopy. The target sample size (1845) was powered to answer the research questions in both parts of the study.

QIAGEN (formerly Digene Corporation, Gaithersburg, Maryland), the Center for Aids Research (CFAR), and Duke University (Durham, North Carolina) (AI064518) provided materials and funding, respectively. The study was approved by both American-based (Duke University Medical School) and Haitian-based (Family Health Ministries) Institutional Review Boards to ensure cross-cultural ethical standards were met.

5.3 Recruitment and Sampling

Women were recruited through clinic referral and word-of-mouth. The clinic administrator at Samaritan's Purse Clinic on Haiti Outreach Ministries' Cite Soleil Compound regularly advertised the study to her staff and patients, and helped

coordinate weekly, free transportation from the compound to Blanchard Clinic for interested participants. Additionally, members of a cancer survivors' support group, Groupe de Support Contre le Cancer (GSCC), encouraged participation by informing friends and church audiences of the study's existence and potential importance to Haitian women's health. Occasionally, Blanchard clinic staff recruited participants from Terre Noire, the neighborhood in which Blanchard Clinic resides, by speaking through a megaphone and addressing passers-by. The staff at CHITAI recruited participants through conversation during their regular gynecological appointments.

These techniques helped successfully recruit and enroll 1836 women who met the inclusion criteria. Specifically, women between the ages of 25 and 65 years old who had engaged in vaginal intercourse at least once during their lifetimes were included in the study. Exclusion criteria were current pregnancy, prior hysterectomy, or active menstruation. Women who came to the clinic while on their periods were asked to return for testing after a couple of weeks.

5.4 Education and Consent

At Blanchard clinic, a trained health worker educated the women as a group in issues related to prevention, transmission, and treatment of cervical cancer and HIV. The danger and likelihood of HIV-HPV co-infection was discussed. Study protocol and procedure was described, including how HPV and/or HIV positive cases would be

handled. Participants were given pamphlets printed in Haitian Kreyol that highlighted key facts from the lecture. Informational posters also decorated the waiting room. At CHITAI, participants were similarly educated and provided with pamphlets, although they interacted with health workers individually rather than as a group.

Following education, participants signed identical Haitian Kreyol and English consent forms in private rooms. Women who could not write their names gave their consent by signing with "X"s. Health workers also obtained demographic information and healthy histories, which they recorded on forms with a randomly pre-assigned patient ID numbers. Repeat patients kept their previous ID numbers for medical record-keeping purposes. All patients were given blue ID cards on which nurses later recorded the dates on which patients ought to return to receive HPV results. The blue color of the cards signified participation in this study, contrasting the white ID cards used for non-participants (regular gynecological patients).

5.5 Clinical Procedures and Materials

With few exceptions, participants completed three tests – a self-collected HR HPV screen, a health worker-collected HR HPV screen, and a rapid HIV test— in one clinic visit. All tests were completed using aseptic techniques, and at a level of privacy considered appropriate in Haitian culture. HR HPV positive patients returned on a prescheduled, later date for follow-up investigation with colposcopy and biopsy. They

were treated with cryotherapy in the clinic or referred elsewhere for other treatment, as needed.

5.5.1 Self-Collected and Health Worker-Collected HPV Tests

Vaginal and cervical samples were taken using conic-shaped brushes— the *digene* Cervical Brush (QIAGEN, formerly Digene Corporation, Gaithersburg, Maryland). First, nurses instructed the patients to rotate the brush three times counterclockwise in their vaginas. Being careful to avoid contamination, the brush was placed in a vial with *digene* Specimen Transport Medium (STM) for storage and transport. Vaginal specimens were marked with blue patient ID stickers and the testing date. Patients were then asked to lie in the dorsal lithotomy position for the second screen. After removing excess mucus from the cervical os and surrounding ectocervix with a cotton swab, a physician or nurse took a cervical sample by inserting a clean brush 1 to 1.5 cm into the cervical os and rotating it three times counterclockwise. The sample was placed in an STM-filled vial labeled with a white patient-ID sticker and the date.

5.5.2 Rapid HIV Test

Rapid HIV tests were performed using Alere Determine™ HIV-1/2 Tests (Alere Medical Co. Ltd., Chiba-ken, Japan). The colloidal gold-enhanced, immunochromatographic assay detected HIV antibodies in whole blood taken from finger pricks. Safety lancets were used to puncture alcohol-disinfected skin, and

heparinized capillary tubes (Chase Scientific Glass Inc., Rockwood, TN, USA) were used to collect the blood and dispense it on the test strip's sample pad. Two drops of buffer solution (Chase Scientific Glass Inc., Rockwood, TN, USA) were immediately added to the sample pad to enhance capillary action. Tests were laid flat for fifteen minutes to allow the blood-buffer solution time to migrate to the top of the assay strip.

5.5.3 Colposcopy and Biopsy

Trained physicians performed colposcopies and biopsies on HR HPV positive patients. Visual inspection with magnification of precancerous lesions was performed after applying acetic acid to the cervix. A single biopsy was taken with Kevorkian Biopsy Specimen Forceps (Instrumed (PVT) Ltd., Sialkot, Pakistan) from a visible lesion whose location was recorded in the patient's medical record. In accordance with part two of the ongoing study (CFAR AI064518), the first 50 women with HR HPV positive tests were randomly biopsied in each quadrant of their cervixes, regardless of the presence of lesions. Specimens were stored in vials of formaldehyde with parafilm-secured lids to prevent leakage during shipment to the diagnostic facility.

5.6 Diagnostics

5.6.1 Self-Collected and Health Worker-Collected HPV Tests

Weekly, de-identified HR HPV brush samples were collected from participating clinics and mailed to a pathology lab in America for diagnosis. QIAGEN tested vaginal

and cervical brush samples for 13 high-risk HPV genotypes (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) using the Hybrid Capture ® 2 High-Risk HPV DNA Test ™ (hc2). In the hc2 nucleic hybridization assay, HR HPV DNA hybridizes with specific RNA probes. Hybrids are captured and immobilized by antibodies on a microplate and then reacted with alkaline-phosphatase conjugated antibodies specific to HR HPV DNA. Because multiple alkaline-phosphatase conjugated antibodies bind to a single HR HPV hybrid molecule, the target molecules have substantially amplified signals. As the bound enzymes cleave from their substrates, light is emitted. The Digene Microplate Luminometer 2000 ™ measures the intensity of the light in relative light units (RLU), which convey the presence or absence of HR HPV DNA in the sample. QIAGEN designates an RLU cut-off (RLU/CO) value of one for HR HPV DNA; less than one indicates its absence (a negative test result) and equal to or more than one indicates its presence (a positive test result). The RLU/CO is defined by the average of the RLU of the Positive Calibrator multiplied by the correction factor.

5.6.2 Rapid HIV Test

Within 15 minutes of test completion, a trained health worker was able to make a diagnosis for HIV. A colored control band appeared on the lower end of the test strip regardless of the test result to indicate that the colloidal gold conjugate assay was functioning. The appearance of a second colored band higher up on the test strip denoted a positive result (100% sensitivity relative to enzyme immune assay (EIA)

results). Positive test results were confirmed with a repeat test on the same day. The absence of a test band denoted a negative result (99.75% specificity relative to EIA results).

5.6.3 Biopsy

Approximately every three to four weeks, biopsy specimens were mailed to PathForceDx (Silverdale, WA, USA) for diagnosis. PathForceDx outsourced the technical component of its analysis to Pathology Associates Kitsap County (Bremerton, WA, USA). A whole slide-scanner (Leica SCN400 Scanner) prepared colorful, 3D, digital images of the pathology slides that were uploaded to a secure cloud workspace. ABP board-certified pathologists examined the histopathologic specimens using the Simagis Live Slide Image Viewer and reported diagnoses as either normal, HPV cytopathic effect, CIN I, CIN II, CIN III or invasive cancer.

5.7 Follow-Up and Treatment

After completing the HR HPV tests, nurses informed participants verbally, and in writing, of the dates on which they ought to return for their results. The health worker performing the HIV tests reiterated the importance of returning for HR HPV results on the scheduled dates. A health worker discreetly informed women of their test results individually. HR HPV negative women were asked to return for follow-up screening in a year, while HR HPV positive women were scheduled for colposcopy and

biopsy within the next few months. Nurses contacted women who failed to return for results and/ or follow-up appointments within approximately a week of the missed appointment.

Because loss to follow-up after HPV screening is a known problem in Haiti—reportedly 50% at Family Health Ministries' Leogane Clinic[5]—a "see-and-treat" protocol was offered to women with pre-cancerous lesions who were felt to be at risk of not returning. Haitian doctors are generally more liberal than American doctors in using cryotherapy to treat low-grade disease because of their concern that many patients may be screened only once in their lifetimes. Patients diagnosed with cancer were referred to local hospitals for staging, surgery, chemotherapy, palliative care or possible referral out of the country for radiation therapy. Financial assistance for some patients was secured through GSCC. The flow chart in Appendix A outlines FHM's cervical cancer screening and treatment protocol.

HIV positive women (confirmed with a repeat test during the same visit) received post-test counseling from trained health workers immediately after results were available—typically within 15 to 30 minutes. Women were referred to local organizations—Groupe Haitien d'Étude du Sarcome de Kaposi et des Infections Opportunistes (GHESKIO) and Fondation pour la Santé Reproductrice et l'Education Familiale (FOSREF)— for free, follow-up care, including anti-retroviral treatment.

5.8 Confidentiality

United States' Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. As required by these laws, study records with identifying patient information have been kept private. Family Health Ministries linked study records to patient records using uniquely assigned code numbers. The key to deciphering the code is stored in a locked file in the research office at FHM's headquarters in North Carolina.

Study participants are aware that representatives of the National Institutes of Health or Duke University Health System's Institutional Review Board may review their records to ensure adherence to federal or state regulations. All study-related records will be retained by FHM for at least six years after the study's completion.

6. Data Analysis

1845 women completed both vaginal and cervical HR HPV screens, though only 1836 were used in analysis because nine women's study-specific consent forms could not be located. Descriptive, demographic statistics were generated using all available data, including statistical outliers. For example, the calculation for mean age of sexual debut included three women whose ages of first sexual encounters were recorded as 4, 8, and 9. At the time of publication, cervical biopsy results were available for 106 women. When all biopsies have been performed, and tissue samples diagnosed, the dataset will include histopathological information for 445 women.

Overall prevalence rates were calculated for HIV, vaginal and cervical HR HPV, and level of disease observed in biopsy samples. Biopsy results among HR HPV positive patients were characterized as HPV cytopathic effect, CIN I, CIN II, CIN III, and invasive cancer. HR HPV prevalence rates were also stratified by age quartiles and tested for significance of difference using chi-square tests. Participants were considered "HPV positive" if they had at least one HR HPV positive test result—either vaginal, cervical, or both.

Overall percent agreement, and strength of this agreement relative to an expected value (measured by a Kappa statistic, K), was determined for self-collected and clinician-collected samples. The Kappa statistic was evaluated on a scale from – 1 to 1, where negative values indicate agreement less than chance (i.e. potential systematic

disagreement between the inter-rater categories), "0" is exactly what one would expect by chance, and positive values signify agreement better than chance [48]. A confidence interval was determined for the Kappa statistic using an analytical method for dichotomous variables that is standard in STATA 12.0 [99]. Strength of HR HPV test agreement was similarly calculated for age percentage quartiles (with ranges of 23 – 34, 35 – 41, 42 – 48, and 49 – 68 years) and biopsy result categories (HPV cytopathic effect, CIN I, CIN II, CIN III, squamous cell carcinoma/ cancer). For patients who were randomly biopsied in four cervical quadrants, the highest level of disease found in any of the quadrants was reported and used for analysis. Relationships between nominal variables were calculated using chi-square or Fisher's Exact tests. The latter was conducted for concomitant HR HPV test results vs. biopsy results because of the small sample size. McNemar's test was used to compare paired sets of data.

All analyses were conducted using STATA 12.0.

7. Results

7.1 Study Population Characteristics

The median age of women enrolled in the study was 46 years. The mean age was 41 years, and the median range of participants (25-75% quartiles) fell between 34 and 48 years. Five women's ages were outside of the inclusion criteria; there were three 23 year-olds, one 24 year-old, and one 68 year-old. 80.6% of participants were married or living with a partner. The majority of women (62.37%) had only one or two sexual partners in their lifetimes, but half (50.76%) of the women's partners had children with other women. On average, women had two or three living, biological children (mean = 2.43, SD +/- 1.87), and made their sexual debuts at 19.78 years of age (SD +/- 4.59). 27.3% of women had experienced at least one miscarriage during pregnancy, while 41.3% had electively terminated at least one pregnancy.

7.2. Test Positivity and Disease Prevalence Rates

High-risk HPV DNA was detected in 21.41% (393/ 1836) of self-collected, vaginal samples and 18.57% (341/ 1836) of health worker-collected, cervical samples. 24.29% (446/ 1836) of women had at least one HR HPV positive test result and were referred for follow-up diagnostic testing. Self-screening identified 88.10% of all women infected with HR HPV in their reproductive tracts, compared to clinician screening, which

identified 76.40%. Assuming 100% sensitivity of HR HPV detection for clinician-screening, self-screening missed disease in 15.54% of cervical positive cases; in other words, vaginal samples identified 84.46% (288/ 341) of positive cases that were identified by cervical samples.

Table 1. Two-by-Two Table of Concomitant HR HPV Results with Percent Frequencies

	Cervical –	**Cervical +**	**Total**
Vaginal –	1390 75.71%	53 2.89%	1443
Vaginal +	105 5.72%	288 15.69%	393
Total	1495	341	**1836**

Vaginal samples garnered higher rates of positivity than cervical samples among all age groups. Both vaginal and cervical HR HPV prevalence decreased with age, although the oldest quartile (49 – 68 years) had slightly higher prevalence than 42 to 48 year-olds. Prevalence rates differed with statistical significance ($p < 0.001$) by age quartile, according to chi-square tests within all three diagnostic categories of interest: vaginal positive, cervical positive, and vaginal or cervical positive. McNemar's chi-square test indicated that prevalence rates garnered by self- and clinician-screenings were significantly different for the youngest and oldest age quartiles, but were statistically comparable in the other categories.

Table 2. HR HPV Prevalence by Age Quartile

Quartile	*Age Range (Years)*	*HR HPV Prevalence (Vaginal +)*	*HR HPV Prevalence (Cervical +)*	*McNemar's $X^2(1)$*	*HR HPV Prevalence (Vaginal + or Cervical +)*
0 – 25% *(n = 490)*	23 – 34	32.24%	27.55%	*9.62 p < 0.01	35.51%
26 – 50% *(n = 435)*	35 – 41	22.53%	21.15%	0.95 p = 0.33	26.21%
51 – 75% *(n = 458)*	42 – 48	13.76%	11.57%	3.33 p = 0.07	15.94%
76 – 100% *(n = 453)*	49 – 68	16.34%	13.47%	*4.83 p < 0.03	18.76%
*Pearson's $X^2(3)$ *statistical significance*		**57.40 p < 0.001*	**50.68 p < 0.001*	*N/A*	**59.30 p < 0.001*

At the time of publishing, 80.90% (360/ 445) of HR HPV positive women had completed colposcopy and biopsy. Histopathology results were available for 33.89% (122/ 360) of completed biopsies. Five HPV negative women were inadvertently biopsied due to staff oversight, and their histopathology results are pending. Of the biopsies with results available to date, 8.20% (10/122) presented with benign mucosa, 39.34% (48/122) with HPV cytopathic effect, 43.44% (53/122) with CIN I, 4.10% (5/ 122) with CIN II, 3.28% (4/ 122) with CIN III, and 1.64% (2/ 122) with cancer.

7.3 Test Agreement

Overall, there was a 91.39% (1677/ 1836) agreement between the concomitant HR HPV tests (K= 0.73 [95% CI: 0.69 – 0.77], SE= 0.02, p < 0.001). This agreement is much greater than the statistically expected value of 68.03%, as evinced by the proximity of the Kappa statistic to "1," which suggests perfect agreement. Kappa statistics within the range of 0.61 – 0.81 are considered to denote "good" strength of agreement[79].

When stratifying HR HPV test results by age quartile, chi-square testing demonstrated that age had a statistically significant ($p < 0.05$) impact on concomitant results' test agreement, and reiterated the connections between age and vaginal results, and age and cervical results. There was a general trend towards greater test concordance with increasing age, although 42 to 48 year-olds had higher concordance (93.45%) than the oldest age group, 49 to 68 year-olds (92.46%). All corresponding Kappa statistics (range: 0.69 to 0.74) indicated statistically significant, substantial strengths of agreement (Table 3). It is unlikely that the observed agreement between test results was due to chance.

Table 3. Concomitant HR HPV Test Agreement by Age Quartile

Quartile	*Age Range (Years)*	*Both –*	*Vaginal – Cervical +*	*Vaginal + Cervical –*	*Both +*	*Observed Agreement*	*Kappa Statistic [95% CI]*
0 – 25% *(n = 490)*	23 – 34	316	16	39	119	88.78%	0.73 [0.67 – 0.80] p < 0.001
26 – 50% *(n = 435)*	35 – 41	321	16	22	76	91.26%	0.74 [0.67 – 0.82] p < 0.001
51 – 75% *(n = 458)*	42 – 48	385	10	20	43	93.45%	0.70 [0.61 – 0.80] p < 0.001
76 – 100% *(n = 453)*	49 – 68	368	11	24	50	92.27%	0.69 [0.60 – 0.79] p <0.001
Pearson's $X^2(3)$ **statistical significance*						**7.19* *p* < 0.001	

7.4 Detection of CIN

Vaginal brush samples identified 90.63% of women known to have ≥ CIN I (Table 4), and 90.91% known to have ≥ CIN II (Table 6). Vaginal screening alone would have missed six cases of CIN I and one case of CIN II. Cervical brush samples performed slightly better by detecting 95.31% of patients with ≥ CIN I (Table 5), and 100% with ≥ CIN II (Table 7). Cervical screening alone would have missed three cases of CIN I. Both tests detected 100% of CIN III and cancer cases. Detection rates of ≥ CIN I were comparable (p = 0.74) according to a Fisher's Exact Test, which was unable to be

performed on a two-by-two contingency table of ≥ CIN II detection rates due to an insufficient number of observations in each of the cells.

Similarly, for the individual histopathological categories, p values could only be generated for the HR HPV test results of patients with HPV Cytopathic Effect and CIN I. The tests of association conveyed that there were no statistical differences in the detection rates of vaginal and cervical tests within the two histopathological categories (Table 8).

Table 4. Two-by-Two Table of Self-Collected, Vaginal Samples' HR HPV Results vs. ≥ CIN I Diagnosis

	< CIN I	≥ CIN I	Total
Vaginal –	5	6	11
Vaginal +	53	58	111
Total	58	64	**122**

Table 5. Two-by-Two Table of Clinician-Collected, Cervical Samples' HR HPV Results vs. ≥ CIN I Diagnosis

	< CIN I	≥ CIN I	Total
Cervical –	16	3	19
Cervical +	42	61	103
Total	58	64	**122**

Table 6. Two-by-Two Table of Self-Collected, Vaginal Samples' HR HPV Results vs. ≥ CIN II Diagnosis

	< CIN II	≥ CIN II	Total
Vaginal –	10	1	11
Vaginal +	101	10	111
Total	111	11	**122**

Table 7. Two-by-Two Table of Clinician-Collected, Cervical Samples' HR HPV Results vs. ≥ CIN II DIagnosis

	< CIN II	≥ CIN II	Total
Cervical –	19	0	19
Cervical +	92	11	103
Total	111	11	**122**

Table 8. Concomitant HR HPV Tests' Detection of Histopathology

Histopathologic Diagnosis	*Both –*	*Vaginal – Cervical +*	*Vaginal + Cervical –*	*Both +*	*Vaginal Test Detection*	*Cervical Test Detection*	*Fisher's Exact Test*
Benign Mucosa *(n = 10)*	--	--	8	2	100.00%	20.00%	--
HPV Cytopathic Effect *(n = 48)*	--	5	8	35	89.58%	83.33%	0.38
CIN I *(n = 53)*	--	5	3	45	90.57%	94.34%	0.74
CIN II *(n = 5)*	--	1	--	4	80.00%	100.00%	--
CIN III *(n = 4)*	--	--	--	4	100.00%	100.00%	--
Cancer *(n = 2)*	--	--	--	2	100.00%	100.00%	--

8. Limitations

Several issues related to biopsy collection and diagnosis limited the analysis and interpretation of the histopathology dataset. At the time of publication, the dataset was incomplete: 360/ 445 HR HPV positive women had been biopsied, and 122 had results. Without a robust sample size that included more than a few women with ≥ CIN II, it was difficult to assess with certainty the vaginal and cervical screens' abilities to identify women with high-grade disease.

This assessment might also have been hampered by the time lapse between screening for HR HPV and performing colposcopy and biopsy. For the 360 HR HPV positive women who have been biopsied to date, the average amount of time between their HR HPV test and biopsy was 61 days. 25% had more than a 6-month time gap between initial and follow-up visits. During the time between screening and undergoing biopsies, it is possible that some women's infections cleared, while others' advanced. If an infection digressed or progressed enough to alter the viral load of HR HPV DNA in the reproductive tract (which corresponds to hc2 signal strength that is used to determine positivity), the initial test results could be an inaccurate reflection of the woman's HR HPV status at the time of biopsy.

However, some scientists might contest this idea. Schiffman and colleagues (2011) suggest that waiting an unspecified amount of time between primary screening and colposcopy could actually improve accuracy over same-day biopsies. They contend

that CIN III lesions are initially difficult to spot because of their small size, leading clinicians to inadvertently miss high-grade disease[100]. Indeed, colposcopies are a highly subjective screening method. Current guidelines direct colposcopists to biopsy the "most worrisome" visible lesion, but intercolposcopist agreement upon where the biopsy ought to be taken is mediocre. In this study, two clinicians, trained in different countries, performed the procedure[101-102]. Studies show that single colposcopic examinations miss approximately one third of CIN II and III lesions[103,104]. Increasing the number of biopsies taken does improve sensitivity, however, regardless of the experience of the colposcopist[105,106,107]. Therefore, it is possible that subjects who underwent four-quadrant biopsies received more sensitive results than those who were biopsied only once from their most suspicious lesion. In fact, data analysis on the incomplete dataset of multiple, random biopsies does indicate the presence of high-grade disease in samples with healthy appearance.

The pathologists who were not blinded to the HR HPV results introduced additional bias. Knowing the vaginal and cervical results could have influenced them to downgrade their diagnoses for women who tested negative for cervical HR HPV, but positive for vaginal HR HPV. Branding true positives as false positives could have reduced the vaginal test's sensitivity for clinically-relevant disease. All ten diagnoses of "benign mucosa" were associated with samples that were positive for vaginal HPV, while only two were positive for cervical HPV. Global research has demonstrated that

the pathological interpretation of cervical biopsies is sometimes subjective, documenting poor inter-observer and intra-observer reproducibility of diagnoses of CIN II and III[36].

Lastly, this study's results could have been enhanced by the inclusion of sensitivity and specificity calculations for both cervical and vaginal samples. A screening method's sensitivity and specificity are fundamental to its selection as a useful tool for cancer prevention, as they reveal information needed to estimate testing intervals and choose secondary or triage tests. Without a universally applied reference standard in this study—only HR HPV positive women were assessed by colposcopy and histology—this information was incalculable. The absence of a reference standard diminished the understanding of the concomitant screening tests' power of disease detection, and though unlikely, might have denied at-risk women (who received false negative HR HPV results) the follow-up care they needed.

9. Discussion

To assess the generalizability of results, and to place this discussion in the appropriate context, it was important to compare this study population to the broader Haitian and Caribbean/ Latin American populaces. In general, the makeup of this sample from Port-au-Prince appears to closely represent the overall Haitian, adult, female population.

The World Health Organization (WHO) and Institute Catala d'Oncologia (2010) estimated that the median age of first sexual intercourse among Haitian women currently between the ages of 25 and 49 years is 18.1 years old. Within the same age bracket, members of this study population had a median age of sexual debut of 18, and a mean of 19.5 years. HIV prevalence in this population (2.23%) also matched the latest WHO countrywide estimate for adults between the ages of 15 and 49 years (2.20%). As one would expect, the prevalence rate among participants (most of whom had only one or two lifetime sexual partners) was considerably lower than estimates for female sex workers in Port-au-Prince (5%)[108].

According to the Haitian Demographic and Health Survey (2000), 55% of men engaged in extramarital sex within the last year. While respondents were not asked specifically about their partners' faithfulness (due to the cultural complexity of the issue), they were asked to report if their partners had children with other women. 51% responded affirmatively. Although a direct comparison between these statistics cannot

be made, the numbers are related, particularly given that contraception is not widely practiced in Haiti. Only 3.3% of all Haitian women use oral contraception, and 24.8% of married women use some form of modern contraception[108]; therefore, it is likely that many extramarital relationships result in pregnancy.

More than 99% of study participants reported affiliation with a particular religion. Most identified themselves as Protestant (60.84%), Catholic (19.61%), or Christian (16.94%)—the remaining described themselves as Mormons or Jehovah's Witnesses. While the term "Christian" can refer to a follower of any Christian denomination, in Haiti, Protestants more often espouse the label than Catholics. With or without considering self-identified Christians as Protestants, the prevalence of Protestantism among the national female population (53%) is significantly lower than among study participants, while Catholicism (39%) is significantly higher—in fact, double—than among study participants [109]. Given that women were actively recruited from local Protestant churches, this skewed religious distribution is unsurprising. However, as religious affiliation is of unknown association with HPV or its risk factors in this study or the broader Haitian, it is impossible to draw conclusions about external validity based on this variable.

Table 9. Cervical Cancer Risk Factors: Study Population vs. National Population

Cervical Cancer Risk Factors	*Study Population (2012)*	*Haitian Population (2010)*
HIV Prevalence	2.23%	2.20%
Age of Sexual Debut	18 years	18.1 years
Male Partner has Additional Female Partner	51%	55%

9.1 HR HPV Self-Screening's Diagnostic Performance

9.1.1 Test Agreement

The results generated from this study portend a promising future for vaginal, self-screening in Haiti. By all measures, the prevention method scored on par with the highly sensitive, established technique of cervical, clinician-performed screening. Two of the three hypotheses subtending the primary research objective came to fruition, while the third fell short by less than five tenths of a percentage point. In manifesting these predictions, self-screening in Haiti equaled or rivaled its diagnostic performance in other settings.

Displaying 91.39% concordance with cervical samples, vaginal samples exceeded the statistical expectations for chance agreement alone (68.03%) with a strong Kappa value (0.73). These numbers almost identically match those from previous studies in

Brazil, Germany, China, and India[56,64,87,110], and demonstrate an improvement over those recorded by researchers in Gugulanthu, South Africa and Munich, Germany[84,86]. The consistent, diagnostic performance of self-sampling against clinician-sampling augments its broad, cross-cultural appeal as an alternative to more invasive methods of screening.

9.1.2 Detection of Cervical Positivity

It was hypothesized that vaginal samples would contain HR HPV in at least 85% of positive cervical cases, and the reality came close at 84.46%. This number is not very informative on its own, however. To determine the significance of the discrepancy, one must examine the histopathological characteristics of the women who tested negative for HR HPV in the vagina but positive in the cervix. 53 women fit this criteria, and of those, 11 have biopsy results: five were diagnosed with HPV cytopathic effect, five with CIN I, and one with CIN II. Because low-grade disease most often corresponds to transient infection, it is generally of little concern to clinicians. However, in Haiti, because women are rarely screened, CIN I lesions are sometimes preemptively treated with cryotherapy. In order to catch CIN I in the future, pathologists might consider adjusting the RLU/CO cut-off value which, at 1 pg/mL, is designed to identify cases of ≥ CIN III with near-perfect sensitivity. If the RLU/CO cut-off value were adjusted to 0.5 pg/mL for vaginal samples, self-screening would have identified one more case of disease; if it had been adjusted to 0.4 or 0.3 pg/mL, three to five more cases of disease would have been identified, respectively. With a dataset of only 11 biopsy results in this category, it is

difficult to interpret the benefit of a lower cut-off value at this time. Based on other study's findings, it would be prudent to re-examine this issue once the dataset is complete.

9.1.3 Detection of CIN

Although demonstrating comparability to an inveterate test is important to proving functionality, affirming an ability to detect pre-cancerous and cancerous disease is salient to proving efficacy. Of biopsy-confirmed cases of ≥ CIN I and ≥ CIN II, vaginal samples identified HR HPV in 90.63% and 90.91% of them, respectively. A Fisher's Exact Test revealed that cervical samples' detection rate was not significantly higher for ≥ CIN I ($p=0.74$); though a test of association could not be conducted on ≥ CIN II samples due to inadequate sample size, cervical samples led vaginal samples by 9%. However, it is likely that the gap will narrow with more cases of high-grade disease based on the currently available data: self-screening identified four of the five women with CIN II, and 100% of those with CIN III and cancer. While the detection rates from this study cannot be equated to the sensitivities reported in others (due to the absence of a universally-applied reference standard), they do suggest powerful diagnostic capability.

9.2 Age-Related Statistics

9.2.1 Prevalence Rates

To imbue test results with greater meaning, age-related statistics were generated, as the links between age, HPV prevalence, and hc2 test performance are well-documented. As hypothesized, HR HPV prevalence rates (overall, vaginal, and cervical) varied with statistical significance by age quartile, with the youngest (23-34 years) bearing the greatest burden and the middle-aged (42-48 years) bearing the least. As has been observed in other countries in the Latin American region (Brazil, Costa Rica, Chile, Colombia, and Mexico) and in Holland, a second peak in prevalence occurred in the oldest age cohort (49-68 years)[11,88,111].

Three explanations may be offered for this dilatory spike in prevalence, though all suggest hc2 test mal-performance. Bacterial infections and LR HPV, the latter especially known to affect post-menopausal women more than other age groups, sometimes cross-react with the hc2 probe to cause false positive results[88,112]. Additionally, douche and vaginal jellies are believed to impact results[14]. It has been reported in studies from Little Haiti, Miami, that Haitian women douche several times a day as part of ingrained tradition, particularly out of concern for vaginal muscle tone[43,45]. It is possible that older women engage in this practice more often than younger women, thus rendering their HPV tests more vulnerable to chemical interference. Furthermore, because hygiene conditions are poor in Haiti, and douching products used are both

natural and synthetic, it is possible that douching may be responsible for the high prevalence of inflammatory reproductive tract infections – again, a possible source of hc2 cross-reactivity[113,114]. Although it is impossible to discern the source of the second prevalence peak from this study's data alone, there is a compelling case to investigate HR HPV *and* LR HPV genotypes in the Haitian population. Future community-based trials would also benefit from incorporating an educational component that cautions women of the dangers of frequent douching, or at least the need to desist for several days before submitting a vaginal sample for HR HPV testing.

9.2.2 Test Agreement

Interestingly, prevalence rates not only vary by age, but by testing location. While vaginal and cervical samples' positivity were comparable among the median 50% of subjects, they differed with statistical significance among the youngest and oldest quartiles. There are a couple of possible explanations for the difference in vaginal and cervical specimens of 23 – 34 year-olds: new sex partners and incident infections. One study showed that HR-HPV is associated with vaginal samples in the first four months after intercourse with a new partner, but is only associated with cervical samples at least five months after sex with a new partner[9]. Because young women may be more likely to have had sex recently, and with a new partner, than older women, it makes sense that they would have higher vaginal positivity than cervical positivity. Marriage rates among the youngest cohort compared to the others corroborates this assumption that

older women are more likely to be monogamous. Young women are also more likely than older women to experience incident infections that clear before migrating from the vulvovaginal area into the cervical tissue[115].

Possibly impacting the results of both the youngest and oldest women in the study is the globally-documented increased prevalence of LR HPV in these age groups. LR HPV, which can cross-react with hc2 to produce false positive results, has been found to occur more often in the vagina than in the cervix[86,88]. In fact, among 450 women at community health clinics in South Africa, LR HPV was twice as common in self-collected samples than clinician-collected samples[86]. These statistics reiterate the need for HPV genotyping in Haiti and age-adjusted hc2 cut-off values that accommodate for these possibilities.

10. Conclusion

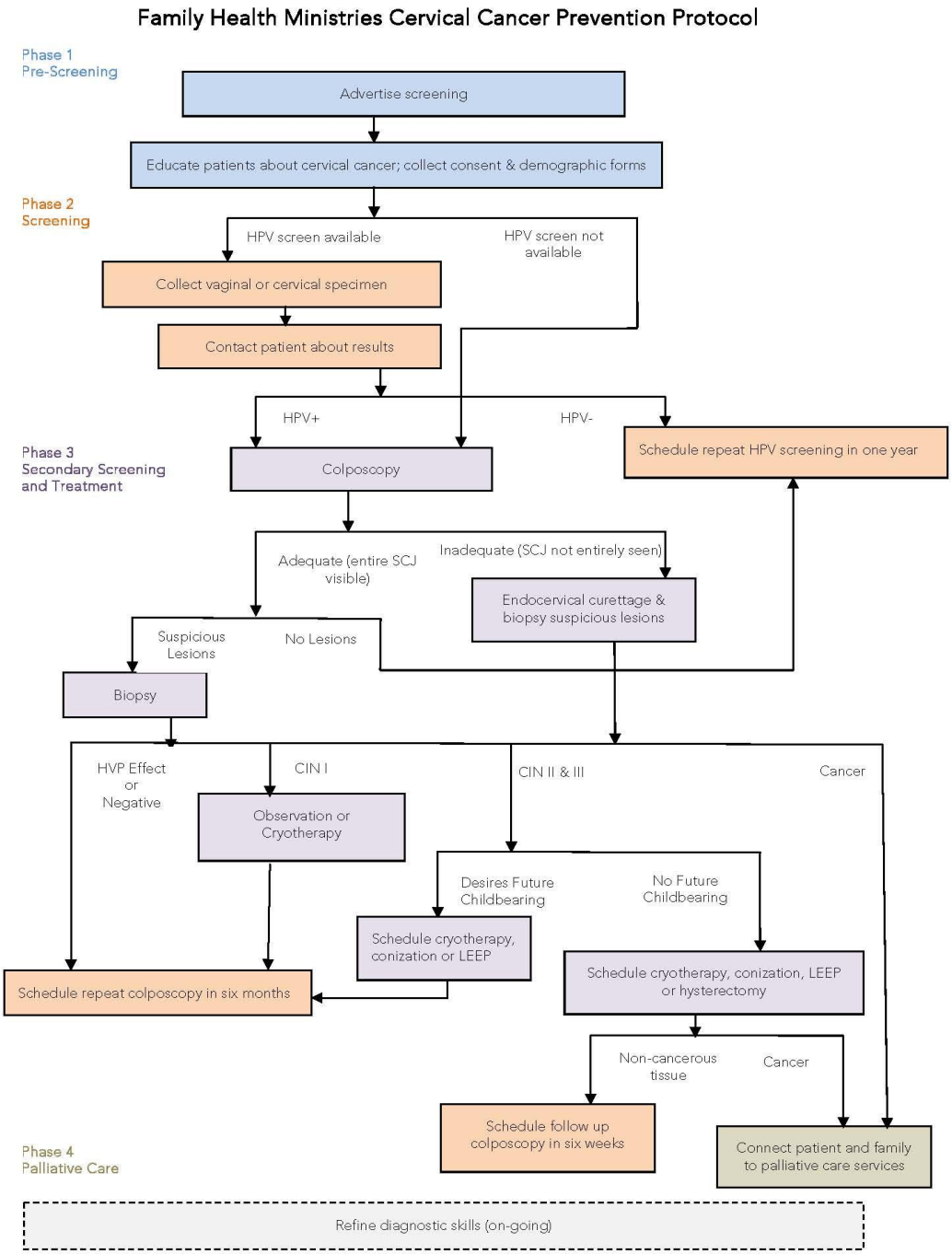
Cervical cancer should be a vestige of a bygone era in which prevention strategies like screening, vaccinations, and early treatment of pre-cancerous lesions were yet to be conceived. Instead, the disease persists as one of the most common and deadly to women worldwide, though disproportionately to those in low-income countries. Haiti, a nation with scant health data and a negligible national budget, lacks a cervical cancer screening program despite possessing the highest reported cervical cancer incidence and mortality rates in the region. With numerous financial, geographic, and cultural barriers to the health system, screening coverage rates have remained abysmally low. Based on acceptability studies in Little Haiti, preliminary research by Partners in Health, and the data presented in this paper, it appears that self-screening for HPV is a suitable approach to curbing the incidence and mortality of cervical cancer in Haiti.

In accordance with reports from other low-resource settings, vaginal self-screening in Port-au-Prince demonstrated strong diagnostic ability. The method proved highly concordant with cervical, clinician-performed screening, and demonstrated an ability to detect the preponderance of known cases of high-grade disease. Interestingly, data indicate that while hc2 test performance is strong for women of all ages, vaginal and cervical results can disagree by a significant margin in women under 34 and above 49. In both cohorts, vaginal positivity was greater than cervical positivity. Previous research supports several explanations involving recent and transient infections and hc2

cross-reactivity with bacteria, vaginal jellies or douche products, and LR HPV. With age-related prevalence data, hc2 cut-off values can be adjusted to accommodate for possible interference. In Haiti, the optimal test would detect all cases of ≥ CIN II and most cases of CIN I. Catching and treating disease early is an advantage in a setting where women rarely interact with the health system and have limited access to surgery or radiotherapy for advanced cancer.

Further research should focus on genotyping both HR HPV and LR HPV in the vagina and cervix. This information would help clarify the potential for hc2 cross-reactivity and the risk for progression of infection. ROC curves can optimize hc2 test algorithms for different populations, taking into account the health and economic results that come with favoring either sensitivity or specificity. In establishing age-specific hc2 detection levels, it could also be useful to explore the relationships between HPV viral load and CIN, and viral load and testing location (lower vagina, upper vagina, and cervix). Interesting hypotheses and data have been generated on the topics[9,86,87], but the body of research is not yet robust enough to be considered conclusive or clinically useful. Armed with more detailed knowledge of HPV test results, colposcopists and pathologists in Haiti could be supported in rendering diagnoses and consequently reducing the overwhelming burden of cervical cancer in Haiti.

Appendix A


Family Health Ministries Cervical Cancer Prevention Protocol
Phase 1
Pre-Screening
Advertise screening
Educate patients about cervical cancer; collect consent & demographic forms
Phase 2
Screening
HPV screen available
HPV screen not available
Collect vaginal or cervical specimen
Contact patient about results
HPV+
HPV-
Schedule repeat HPV screening in one year
Phase 3
Secondary Screening and Treatment
Colposcopy
Adequate (entire SCJ visible)
Inadequate (SCJ not entirely seen)
Endocervical curettage & biopsy suspicious lesions
Suspicious Lesions
No Lesions
Biopsy
HVP Effect or Negative
CIN I
CIN II & III
Cancer
Observation or Cryotherapy
Desires Future Childbearing
No Future Childbearing
Schedule cryotherapy, conization or LEEP
Schedule cryotherapy, conization, LEEP or hysterectomy
Schedule repeat colposcopy in six months
Non-cancerous tissue
Cancer
Schedule follow up colposcopy in six weeks
Connect patient and family to palliative care services
Phase 4
Palliative Care
Refine diagnostic skills (on-going)

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